Integrating the Molecular Machines of Mercury Detoxification into Host Cell Biology

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The Materials Flow of Mercury in the Economies of the United States and the World

By John L. Szeppek and Thomas G. Brenan

U.S. Geological Survey Circular 1197
All forms of Hg are biologically available.
Cysteine (Cys, C)
## Potential Human Targets for Interaction with Hg(II)

<table>
<thead>
<tr>
<th>System</th>
<th>Protein/Process</th>
<th>Molecular Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal transduction</td>
<td>Protein tyrosine phosphatase</td>
<td>Invariant Cys215</td>
</tr>
<tr>
<td></td>
<td>Zinc Finger Proteins</td>
<td>Multiple Cysteines</td>
</tr>
<tr>
<td></td>
<td>L I M proteins</td>
<td>Multiple Cys-His domains</td>
</tr>
<tr>
<td>Metal Homeostasis</td>
<td>Metallothione i n</td>
<td>Multiple Cysteines</td>
</tr>
<tr>
<td></td>
<td>Menkes Disease (Cu)</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Wilson's Disease (Cu)</td>
<td>&quot;</td>
</tr>
<tr>
<td>Renal transport</td>
<td>CHIP28 Water Channel</td>
<td>Cys 189</td>
</tr>
<tr>
<td>Growth Factors</td>
<td>Trefoil, EGF-like, Cystine Knot</td>
<td>Three clustered cystine bridges</td>
</tr>
<tr>
<td>CNS</td>
<td>Membrane Cysteine String Proteins (synaptic vesicles and termini)</td>
<td>Cysteine rich proteins</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>apolipoprotein(a)</td>
<td>Cys 4057 - important for assembly</td>
</tr>
<tr>
<td>Virus e s</td>
<td>HIV Tat protein</td>
<td>Cysteine-rich protein</td>
</tr>
<tr>
<td>Oncogenes</td>
<td>RAS</td>
<td>Thioether farnesyl linkage</td>
</tr>
</tbody>
</table>
Why study Hg resistance?

Only naturally occurring system that biotransform a toxic metal in bulk

Handles inorganic and organic Hg(II)

Widely found in eubacteria and archaea that are the major Hg transformers in highly contaminated settings.

Transposable and laterally transferrable in proteobacteria.

Highly conserved mechanistically - i.e pump Hg(II) in and reduce to volatile Hg(0)

Illuminates some basic biology of enzymology, gene regulation, redox metabolism

Employed in paradigm example of engineered metallophytoremediation
Transgenic *merA* tobacco plants survive transplantation to contaminated soils and detoxify Hg(II) to less toxic Hg(0)

<table>
<thead>
<tr>
<th>Hg(II)</th>
<th>0 ppm</th>
<th>100 ppm</th>
<th>500 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>merA</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GA Piedmont 2% organic

GA Coastal 2% organic


Poster, Weds night
Jeffra Schaefer, Rutgers

**Gram +**
- Bacillus megaterium
- Bacillus cereus, Clostridium butyricum
- Staphylococcus aureus pI258
- Streptomyces lividans
- Streptomyces pRJ28
- Exiguobacterium sp.

**Gram -**
- Pseudomonas sp. ED-23
- Pseudomonas stutzeri OX pPB
- Pseudomonas sp. K62 pMR26
- Serratia marcescens pDU1358
- Pseudomonas aeruginosa Tn501
- Shigella flexneri Tn21
- Alcaligenes pMER610
- Pseudomonas sp. ADP
- Xanthomonas campestris Tn5044
- Xanthomonas sp. Tn5053
- Pseudomonas fluorescens
- Shewanella putrefaciens pMERPH
- Thiobacillus ferrooxidans
- Pseudoalteromonas haloplanktis

Legend:
- merR
- merG
- merT
- merP
- merC
- merE
- merA
- merB
- merD
- 1000 bp
The Bacterial Mercury Resistance Locus

MerA, curiously chimeric oxido-reductase

MerR, a mechanistically novel regulator
Hg(II) provokes MerR to underwind the MerO dyad center

MerR’s “muscular” transcriptional control

Using mutants to dissect the mechanism of metal specificity

MerR and MBD bind metals other than Hg in vitro and in vivo, possibly with differing specificities.
MerR binds other thiophilic metals in vivo and in vitro so its specificity as a transcriptional activator must lie in more than just metal binding....

Possibilities:

Other metals do not provoke DNA distortion

YES, Chuan He, U. Chicago, JACS 2004

Other metals don’t bind MerR when it is bound to DNA

NO, Song et al., JMB 2007, in press

Does Hg(II) provoke a conformational change distinct from that of non-inducers?
\(^{19}\text{F NMR: Watching MerR’s Tyrosines}\)

Tyrosine

\[
pK_a \quad 10.05 \pm 0.04
\]

2-Fluorotyrosine (2-FY)

\[
pK_a \quad 9.04 \pm 0.03
\]
A Candidate Allosteric Signalling Pathway in MerR

B

\[ \begin{align*}
\alpha_1 \text{MENNLENNL} & \text{TIGVFAKAAGVN} \text{VETIRFYQRKGLLRE} \\
\alpha_2 \text{PDKPY} & \text{GSIRR YGEADVVRKFVKSAQRLLDE} \\
\alpha_3 \text{IAELLRL DDGTHCEEASSLAEHKLKD} & \text{VREE KMA DL} \\
\alpha_4 \text{ARMETVLSELVCA C} & \text{HARKGNVSCP LIASLQGEA} \\
\alpha_5 \text{GLARSAMP} & \\
\alpha_6 & \\
\end{align*} \]
Using substitution mutants to assign resonances
MerR only
Hg/MerR$_2$ 1.0
Cd/MerR$_2$ 1.0
Zn/MerR$_2$ 1.0
MerOP DNA binding produces large chemical shift changes in Y27 and Y46 of wildtype MerR and mutant Y40E
Metal-specific changes occur at Y27 and Y46 when MerR is bound to MerOP

So DNA constrains MerR’s response to each metal….

…and C82Y in the metal-binding site ‘notices’ DNA binding.
A Candidate Allosteric Signalling Pathway in MerR

B

\[ \alpha_1 \text{MENNLN} \text{TIGVFAKAAGVNVET} \alpha_2 \text{FYQRKGLLRE} \]
\[ \text{PDKP} \text{YGSIRR} \text{YGEADVVRKFVKSAQRL} \text{GFSLDE} \]
\[ \alpha_3 \text{IAELLRLDDGTHCEEASSLAEHKLDVREKMADL} \]
\[ \alpha_4 \text{ARMETVLSELVCA} \text{CHARKGNVS} \text{CPLIASLQGEA} \]

GLARSAMP
One or two repeats of a domain that is homologous to small proteins that bind soft metals such as Cu$^{+1}$, Zn$^{+2}$, Hg$^{+2}$

Typical Structural Components of MerA

Multidomain flavoprotein homologous with glutathione reductase, obligate dimer

cysteines

NmerA

Tn501
AA 1-69

Tn501
AA 96-561

NADPH

FAD

Hg

Tn501
AA 70-95

flexible linker

Catalytic Core
C-terminal CC Remove High Affinity RS⁻ Ligands

Reduction occurs from here

inner complex

interdomain complex

outer complex

middle complex
NmerA Facilitates Transfer from Hg-Thioredoxin in vitro

\[ k_{\text{cat}}/K_{\text{MHg-TRX}} = 3.0 \times 10^4 \, \text{M}^{-1} \, \text{s}^{-1} \]

\[ K_{\text{MHg-TRX}} \sim 300 \, \mu\text{M} \]

\[ k_{\text{cat}}/K_{\text{MHg-TRX}} = 6.0 \times 10^3 \, \text{M}^{-1} \, \text{s}^{-1} \]

\[ K_{\text{MHg-TRX}} \sim 1200 \, \mu\text{M} \]
Potential Modes of MerB/MerA Interactions

A) Transient interaction with direct transfer to Core C-terminal cysteines

B) Transient interaction and transfer to NmerA only

C) Stable complex with Core but transfer facilitated by NmerA

- Cys-S(H)
- Hg(II)
**NmerA Facilitates Transfer from Hg-MerB**

Consistent with Models B &/or C
Coming Attractions !!
Bacterial cell contents to scale.
The Mercury Shock Proteome -- With and without the *mer* Operon

Mary Lipton

Judy Wall, *Desulfovibrio*

Tom DiChristina, *Shewanella*

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University of Missouri-Columbia

Georgia Tech